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The Determination of Malic Acid in Maple Sirup by an Ion Exchange Procedure: An Adaptation of the Goodban and Stark Method*

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The purity of maple sirup is based upon analytical data which include conductivity values, malic acid values, lead numbers, ash (soluble and insoluble), and the alkalinities of the ashes. In screening large numbers of samples, only two of these analyses are used—conductivity values and malic acid values. Unfortunately the available methods for malic acid (1, 3, 5, 6, 8) have not been too satisfactory since they were time consuming or low in specificity. The current A.O.A.C. method (7) is nonspecific, since any acid in the sirup that forms a calcium precipitate insoluble in 85% alcohol is measured as malic acid.

Recently Goodban and Stark (4) reported a rapid method for the determination of malic acid in plant juices. Because of the similarity of maple sirup to plant juices, it appeared that their method might be applicable to the analysis of maple products. This method depends upon (*a*) the removal of cation material from the test solution with a cation exchange resin; (*b*) the absorption of the acids, including malic, on an anion exchange resin; (*c*) the eluting of malic acid as a fraction free of other acids that may interfere in the subsequent color test; and (*d*) the determination of the malic acid spectrophotometrically in a strongly acidified solution after the addition of the color reagent, 2,7-naphthalenediol.

Goodban and Stark showed that certain acids, including succinic, one of the principal acids of maple, might interfere with the test for malic acid if present in large quantities. They also showed that sugar phosphates would interfere with the test. They did not include in their study the applicability of their method to the analysis of malic acid when it occurs with large amounts of sugars, ratio 200 to 1, nor did they show whether or not citric or succinic acids are sufficiently separated from malic acid by the anion exchange resins. Nor is it known what might be the effect of the minor acids of maple sirup on the determination of the malic acid. The present studies were therefore made to provide this information.

Ion-exchange Procedure

(The procedure is based on method developed by Bryant and Overell (2); essentially same as that used by Stark and Goodban (9) to determine lactic acid.)

Grind Dowex 50 resin (cation exchanger) and IRA 400 resin (anion exchanger) to 60–80 mesh and make up in water slurry. Transfer 10 ml portions of each resin to glass ion-exchange columns, 10 mm i.d. ×30 cm long, with 5 cm capillary tip and glass wool plug to retain resins. Condition resins as follows: To Dowex 50, add three or four 10 ml portions of 5% HCl and let acid drain to top of resin between each addition. Wash resin free of acid with 10 ml portions of distilled water until effluent gives no test for chlorides. (Approximately 4 bed volumes of water are required.) Treat IRA 400 with three or four 10 ml portions of 5% NaOH and drain liquid to top of resin between additions. Remove excess alkali by washing with 10 ml portions of distilled water until effluent gives negative alkali *p*H test with indicator paper. Put resin in carbonate form by adding three or four 10 ml portions of Na_2CO_3, and then wash free of excess carbonate with 10 ml portions of distilled water until effluent is neutral to indicator test paper.

Mount conditioned columns vertically with Dowex 50 resin column directly above the IRA 400 column. Add 10–20 ml sample (containing 6–20 mg malic acid) to Dowex 50 column and let pass through freely, letting eluate fall directly into IRA 400 column. (No stopcocks are needed; close packing of fine resin prevents rapid flow of liquid

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and also prevents liquid from draining below resin surface and thus causing drying and inactivation of resins.) Wash upper (cation) column with three 10 ml portions of water, letting effluent drip into IRA 400 column. Remove upper column and wash IRA 400 column with three 10 ml portions of distilled water to remove sugars and any loosely-held acids that may be present and that would interfere with test. Elute column with five or six 10 ml portions of 0.25*N* ammonium carbonate to quantitatively remove all of the glycolic, glyceric, or lactic acids that might have been present in original test solution. Next elute malic acid from resin with five 10 ml portions of 1*N* ammonium carbonate, collect 45–48 ml eluate in 250 ml volumetric flask, remove flask, and dilute eluate to volume with distilled water.

Development and Measurement of Color

Transfer 1 ml of the malic acid-ammonium carbonate eluate to 18 mm×14 cm test tube; add 6 ml 96% H_2SO_4 (analytical grade) slowly, from buret, letting first 2 ml run down walls of tube to avoid excessive evolution of CO_2. Then add 0.1 ml of the 2,7-naphthalenediol reagent and mix thoroughly with strongly acidified malic acid solution. Heat tubes in boiling water bath for 25 min. to develop color. Cool tubes, and measure absorbances of colored solutions on spectrophotometer at 390 mμ, using 1 cm absorption cells, against blank consisting of 1 ml water, 6 ml H_2SO_4, and 0.1 ml of the reagent, heated 25 min. in the boiling water bath. If absorbance is measured in test tubes in which color was developed, determine "*a*" in Beer's Law expression, reduce malic acid added to columns to 6–12 mg, and measure color within 30 min. after it is developed. Then calculate amount of malic acid in sample as follows: $C=(A/ab)\times$dilution factor, where C is concentation of the malic acid in mg/100 ml, A is absorbance at 390 mμ, b is internal cell thickness, and a is absorptivity.

Experimental

To determine whether or not the color developed with malic acid and 2,7-naphthalenediol in a strongly acidified solution follows Beer's Law, a series of malic acid solutions of different concentrations were tested. It was found that for the expression of Beer's Law, $a=A/bc$, a (absorptivity) is constant and equals 87, calculated from measurements made in a Cary recording spectrophotometer at 390 mμ with a 1 cm cell. In measuring the absorbance, A, Eastman Kodak white label L-malic acid, previously dried for 18 hours at 40°C, was used as the primary standard. All subsequent absorbances were measured in a Coleman Universal spectrophotometer with the test tubes in which the color was developed as the absorption cells. The absorbances were measured by the null point method.

The effect of large concentrations of sugar and the presence of both succinic and citric acids on the determination of malic acid are shown in Table 1.

Cane sugar sirup of 66% (the density of maple sirup) containing the added malic, succinic, and citric acids was diluted enough so that 10 ml of the resultant solution contained between 7 and 12 mg of malic acid. The results indicate that malic acid in concentrated sucrose solutions and in sucrose solutions containing succinic and citric acids can be quantitatively measured by the method of Goodban and Stark with reasonably high precision and accuracy.

The method was further tested for its applicability to the analysis of maple sirups by analyzing maple sirups to which known amounts of malic acid were added. Since the acid composition of maple sirups varies with their grade (color) two grades of sirup were used, U.S. Grade AA and U.S. Grade Unclassified, which represent the two extremes of grades. Two different amounts of malic acid were added to each sirup grade to determine if the method can detect slight differences in malic acid content between different sirups. The results of this study are given in Table 2.

These data indicate that the method of Goodban and Stark gives a satisfactory recovery of known amounts of malic acid added to maple sirups and that it is suitable for the analysis of malic acid in maple products. The precision and accuracy appear to be independent of the grade of sirup. The method is of high accuracy as shown by the comparison of the average of the values

Table 1. Recovery of malic acid added to 100 ml of 66% sucrose solutions

Sample No.	Acid Added	Malic Acid Found, g Range Max.	Min.	Av.	S.D.	No. Detns
1	0.2745 g malic	0.2745	0.2670	0.2722	0.0036	5
2	0.2800 g malic	0.2785	0.2720	0.2747	0.0024	5
3	0.0543 g malic	0.0557	0.0527	0.0543	0.0008	10
4	0.4170 g malic+ 0.4730 g succinic+ 0.5335 g citric	0.4175	0.4054	0.4126	0.0043	10

found and the amount of malic acid added, for both the large and small amounts of added acid. The method is also very precise, as shown by the standard deviations and the ranges of the values found.

The method was applied to the analysis of maple sirups selected from three of the commercial grades. The results are given in Table 3. The results of the 10 analyses of each of the three sirups show that the method will detect differences of $\pm 0.02\%$ malic acid with a high precision as indicated by the low standard deviation and the narrow range of values.

A "t" test was applied to determine whether or not there is a significant difference between the values of the means, 0.51 and 0.53%, found for two of the samples analyzed. Since the calculated "t" was found to be 8.08 with a book value of $t_{.01}$ of 2.87, the method is sensitive and reproducible enough to detect small differences in malic acid values.

Discussion

In establishing a, the absorptivity, L-malic acid was used, since that is the form of the acid found in maple sirups. It was noted, however, that freshly prepared aqueous solutions of the acid gave more constant and higher values for a than did solutions that had been prepared one day or more before making the test. This lowering of the absorbance as the acid solution ages may be accounted for by loss of malic acid through esterification or by dehydrogenation of the β-hydroxy acid. This same decrease in absorptivity was also exhibited by D,L-malic acid. Freshly prepared aqueous solutions of both the L and DL gave identical absorptivities when allowed to react with the reagent.

This work confirms the observation of Goodban and Stark that the absorbance, A, of the colored solution resulting from the reaction of strongly acidified malic acid with 2,7-naphthalenediol remains constant for more than 48 hours. This occurs only if it is kept free from air. Therefore, the colored test solution as well as the blank should be transferred to absorption cells that are completely filled and stoppered for accurate work. However, since the absorbance of the colored malic acid solution remains essentially constant during the first half hour after it is developed, the test tubes in which the color was developed may be used as the absorption cells. This latter procedure also avoids excessive contact (oxidation) of the colored liquid with air that occurs when the solution is poured from the reaction tubes into absorption cells.

Table 2. Recovery of malic acid added to two grades of maple sirup

Sample No.	Wt of Sirup, g	Malic Acid Added Grams	Per Cent	Malic Acid Found, g[a] Range Max.	Min.	Av.	S.D.	No. Detns
				U.S. AA Grade				
1	12.37	0.0094	0.08	0.0109	0.0084	0.0094	0.0011	5
2	11.87	0.0094	0.08	0.0119	0.0094	0.0105	0.0009	5
3	12.48	0.0374	0.30	0.0398	0.0372	0.0388	0.0009	10
				U.S. Unclassified				
1	12.56	0.0398	0.32	0.0404	0.0376	0.0391	0.0011	10
2	13.05	0.0134	0.10	0.0142	0.0121	0.0130	0.0006	10

[a] Corrected for malic acid present in sirup.

The sugars in the sirup added to the ion exchange columns must be quantitatively eluted before the fraction containing the malic acid is collected. Failure to do this will result in high and erroneous values, since as little as 0.005% will cause an appreciable absorption at 390 mμ. Under normal conditions, when the sirup is diluted 10-fold before it is added to the cation column, the three 10 ml water washings plus the four 0.25*N* ammonium carbonate washing of the anion column are enough to remove all of the sugars. To safeguard against incomplete elution of the sugars, a pilot test run should be made on a sucrose sirup, diluted in the usual manner and carried through the ion exchangers in the manner described, and 1 ml of the 1*N* ammonium carbonate eluate acidified and treated with the color reagent. This test solution should have an absorbance no greater than that of the blank.

Washing the IRA 400 resin with 0.25*N* ammonium carbonate must be done carefully, since excessive washing may remove some of the malic acid.

Goodban and Stark showed that glycolic acid when present in the test solution will react with the 2,7-naphthalenediol to produce an absorbance at 390 mμ and thus cause erroneous malic acid values. Glycolic acid, one of the minor acids of maple sirup, is, however, quantitatively removed from the IRA 400 resin column by the 0.25*N* ammonium carbonate washes, prior to the elution of the malic acid.

More than 99% of the malic acid is eluted from the anion column by the first three 10 ml portions of the 1*N* ammonium carbonate solution; 75% of the malic acid is eluted by the second of these three portions. Thus the last two 10 ml washes are used to insure the complete elution of malic acid in cases in which the sample contained excessively large amounts of the acid.

The IRA 400 resin column prepared as directed will hold as much as 150 mg of malic acid without danger of incomplete retention of the acid. However, no more than 25 determinations should be made before the resin is regenerated.

Using 10 pairs of the ion exchange columns, one analyst can make 10 complete analyses per day.

Table 3. Analysis of maple sirups for malic acid

	% Malic Acid Found		
	U.S. AA Grade Sirup	U.S. A Grade Sirup	U.S. Unclassified Grade Sirup
Range, Max.	0.52	0.54	0.52
Range, Min.	0.49	0.52	0.50
Average	0.50	0.53	0.51
S.D.	0.010	0.005	0.006
No. Detns	10	10	10

The close agreement between the percentages of malic acid found in the three sirups representing widely different grades suggests that the total malic acid of all pure maple sirups is probably a constant. This is understandable if we consider that maple sirup is a saturated solution of calcium and magnesium malate at the temperature of the boiling sirup. Thus the following method might be useful for the detection of adulterated maple sirups:

METHOD

Reagents and Apparatus

(a) *Ion-exchange Resins.*[1]—IRA 400, 60–80 mesh; Dowex 50, 60–80 mesh.

(b) *Ammonium Carbonate Solutions.*—0.25*N* and 1.0*N*.

(c) 2,7-*Naphthalenediol.*—1 g in 100 ml 96% H_2SO_4 (analytical).

(d) *Apparatus.*—Ion-exchange columns; test tubes; absorption cells; spectrophotometer.

Transfer approximately 10 ml of the sirup to a tared 100 ml volumetric flask and obtain the weight of the sirup to ±0.0002 g. Dilute to the mark with water and transfer 15 ml to the cation exchange resin, Dowex 50, and proceed as under *Ion Exchange Technique* and *Development and Measurement of the Test Color.*

Summary

An ion-exchange procedure based on the method of Goodban and Stark provides a simple and rapid method for the determination of total malic acid in maple sirup. The method is free of interference from other acids that normally occur in maple sirup, and the sugars that would interfere can be

[1] Mention of trade names in this paper does not constitute recommendation by the U.S. Department of Agriculture over similar products not mentioned.

quantitatively eliminated. The accuracy of the method is within $\pm 0.01\%$. If the value of total malic acid is actually a constant, as results indicate, this method would be a useful tool for the detection of adulteration.

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